In-Situ Stabilization of Subsurface Contaminants Using Microbial Polymers

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Introduction

The U.S. is faced with the increasing problem of nuclear and hazardous wastes. Now, more than ever, the irreversible damage caused by those wastes on both the environment and human health has become a major focal point in the public forum. Both private and public enterprises desperately need a new universal technology that effectively neutralizes such adverse effects. These enterprises are either affected by such in-house problems or are assumed the tasks of mitigating these problems through regulatory, removal, or controlling means.

Objective

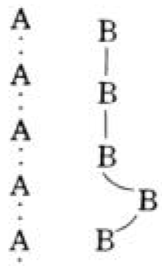
The objective of our project is to apply a new multidisciplinary technology for permanent enclosure and fixation of nuclear and other extreme hazardous wastes in sub-surface sites, specifically Department of Energy sites. The project methodology is centralized on the application of microbial polymers to the subsurface strata to uptake the recalcitrant species of the waste site and effectively fixate the treated soil thereafter. We will develop and test this method of in-situ stabilization of subsurface contaminants using microbial polymers. The simultaneous capture and fixation of the contaminated soil will completely eliminate all hazardous heavy metals, including uranium and plutonium, and isolate the remaining harmful chemicals in such a way that their further release and migration will be eradicated. Our ultimate goal is to store and immobilize all dangerous heavy metals and radioactive materials, and keep them as they are in concentrated small volumes in the subsurface through effective in-situ treatment of the hazardous material.

Approach

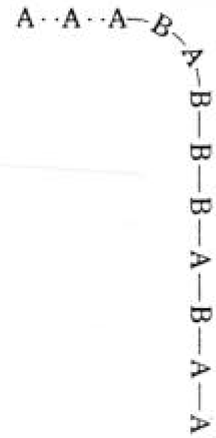
Three types of microbial polymers have been selected for this study, all of which can be found from respective microorganisms. Naturally occurring in nature, microbial polymers are mostly polysaccharides, and are derived from algae, fungus, or other bacterial sources. They can assume different conformations and configurations to fit various functionalities. These microbial polymers can form polycation-polyanion complexes and can also interact with other polymers through cross-linking. For this study, we will use chelating polymers, specifically chitin/chitosan, which are polycationic and mainly derived from the fungal *Mycelia*. The two other types of microbial polymers to be used in this study include bonding polymers, specifically PHB (poly 3-hydrobutyrate), and complexing or cross-linking polymers, specifically xanthan. Bonding polymers are derived from the bacteria *Alcaligenes eutrophus* and, as their name implies, they bond to other polymers. Similarly, complexing or cross-linking polymers bind themselves to other polymers but through different means, such as cross-linking or forming complexes. They are mostly polyanionic and are derived from the bacteria *Xanthomonas campestris*. They are most often used as stabilizing agents.

The main reason for using these three microbial polymers is that they can combine effectively to stabilize soil, which is necessary for this project. To acquire the results desired, these microbial polymers have to complete several processes, such as chelation, bonding, charge complexing, entrapping, etc. The final resulting process will be stabilization, where the end products of the main processes, chelation (C + M? CM) and bonding (P + S? PS), combine with X to form an interpenetrating polymer network (CMPSX) in the reaction (CM + PS + X? CMPSX). Associated processes include charge complexing (C + X? CX), entrapping (CX + P? CXP), interpenetrating network (C + P + X? CXP), and fixation (CXP + M + S? CXPMS), all of which essential to obtain stabilization. In these processes, the microbial polymers can interact with one another in various ways to form different types of polymers. Such types of polymers include polymer blend, random copolymer, block copolymer, alternating copolymer, graft polymer, and interpenetrating polymer network (see Fig. 1).

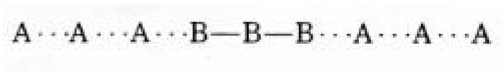
Fig. 1 illustrates the interpenetrating polymer network, an essential step in the stabilization process of soil. An interpenetrating polymer network is the entangling and twisting of two polymers that have the same cross-link or a covalent bond between nonconsecutive parts of each polymer. This is similar to the interaction of a polymer complex that creates polycation and polyanion, and is more tightly bound due to the covalent bonding.



(a) polymer blend

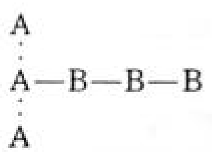


(b) random copolymer

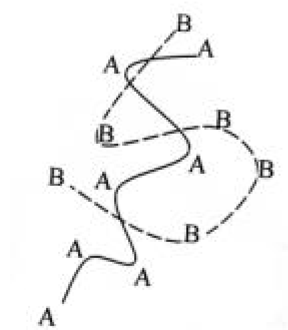


(c) block copolymer

(d) alternating copolymer



(e) graft copolymer



(f) interpenetrating polymer network

Figure 1. Interactions of two different types of polymers, A and B.

Each of the microbial polymers can be derived through the injection of nutrients into the cells or spores allowing the microorganisms to grow. They can be transported in the soil or subsurface environment in the soil strata and migrate. When the microorganisms are dead, or lysed out, the intercellular or external polymer can be released and the three types of polymers will interact. In the future, these microorganisms may be combined into one type of microorganism by genetically altering each of their genes. Since this is a potential development to be dealt with in the future, we have not included further details the present proposal.

The purpose of this project is to apply microbial polymers to the subsurface strata to uptake the recalcitrant species of the waste site and effectively fixate the treated soil thereafter. The simultaneous removal and fixation of the contaminated soil will completely eliminate all hazardous heavy metals, including uranium and plutonium, and isolate the remaining harmful chemicals in such a way that their further release and migration will be eradicated. Three types of microbial polymers to be used are chitin/chitosan, PHB-PHV and xanthan. Chitin/chitosan is a strong metal chelator for a variety of metallic constituents, especially in waste. PHB-PHV is a biopolyester that can form bonds with soil silica and clays. Xanthan, or xanthan gum, is polyanionic in nature and is extremely useful as a stabilizing agent. With these three types of microbial polymers, they form a triple set of interpenetrating polymer networks that involve a polycations-polyanions complex. One side of this complex can bond metal whereas the other side can bond soil particles, such as silica. In such a way, the binding will become trapped in a very stable condition and metal/soil particles cannot be simply removed by leaching. This complex becomes a permanent geopolymer, such as kerogen.

The formation of geopolymer from biopolymer (or biomass) is complex and involves many steps. Biomass is divided into different categorical polymers, such as proteins, carbohydrates, and lipids. Within this structure, these polymers are further divided into specific types, such as amino acids for proteins, hexoses/pentoses for carbohydrates, and polysaturated triglycerides for lipids. The amino acids and hexoses make up the humin/humic acids and combined with the polysaturated triglycerides, they construct an interpenetrating network. Over time, the trapping of biopolymers interacting with metal will eventually form geopolymers, which in this case, form kerogen (see Fig. 2).

Kerogen's structure consists of various integrated structures, such as entrapped species, branched and unbranched aliphatic structures, polymethylene bridges, and a 'cyclic' skeletal carbon structure composed of mostly terpenoids. All of these components are intertwined within one another without conforming to a specific shape or order. This makes kerogen an interpenetrating polymer network.

Geopolymers are used in this project because they are thermally and mechanically stable under many conditions. They do not pyrolyze below 400^{o}C, they are protective and waterproof under conditions of $K<<10^{-12}$ cm/s, they can be used as reactor coolants (in the case of oligophenylenes) and are stable in concentrated acids, such as HF, HCl, and H_2SO_4. Most importantly, metals trapped in geopolymers cannot be leached in the presence of an aggressive liquid in the geosphere. The concept of the accelerated formation of geopolymers used to treat the contaminated sites will assist in stabilizing the metals and preventing the metals from being leached out in aqueous solutions of various pH levels.

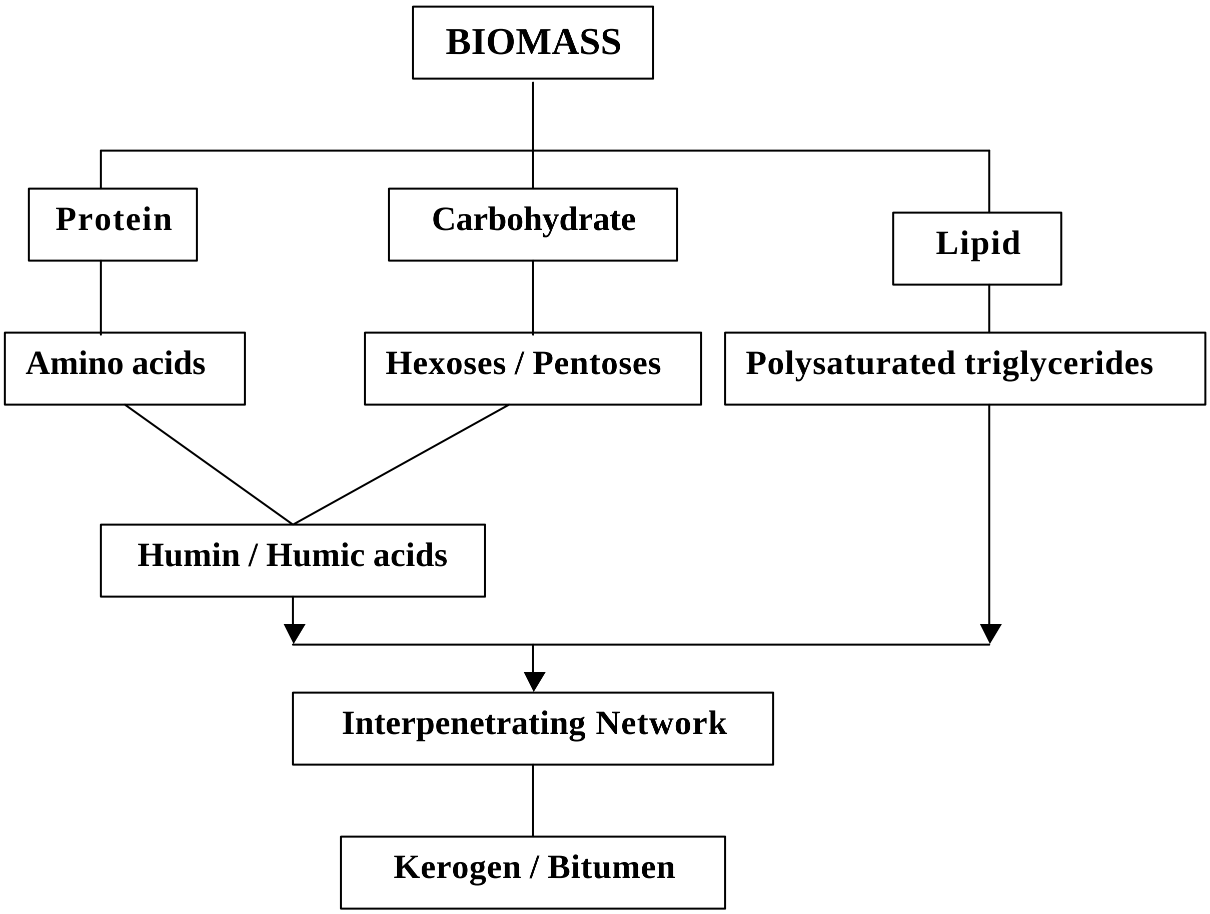


Figure 2. Formation of geopolymer (kerogen) from biomass.

Project Description

This project is separated into two phases. Phase I will be described here, followed by a brief discussion of Phase II. The following tasks are to be completed under Phase I:

Employment of the State-of-the-Art Flow Systems

Flow systems are essential to examine the extent of the stabilization of soil and, in the later stages, to examine the distribution of microorganisms as well as their movement in subsurface strata. In this task, soil sample types will be prepared and studied, and we will evaluate the use of column and sand-pack. We will start with a synthetic medium, using different ratios of the components of soil (clay, sand, and silt) to create representative samples. Using the procedures and setups from our previous work, we will study static drainage flow and pressurized pumping flow. A number of standard soil types with controlled moisture content, pH, metal species, and metal content, as well as three different types of biopolymers (PHB, xanthan, and chitosan) will be acquired for this experiment. Finally, we will add several common metals to the soil medium to determine whether or not the metals are successfully leached or permanently tied to the soil matrix. Torvane tests and calibration will be conducted. The relative permeability, porosity change, soil strength, and metal uptake will be obtained.

Application and Interaction of Biopolymers

In this task, we will identify and analyze the biopolymers in soil, determine whether or not they can form a polyanion-polycation complex and also whether or not they will form and interpenetrating network. If an interpenetrating network is not formed, a cross-linking agent (such as sodium borate or alumina) will be added. We will also determine whether or not the microbial polymers can adhere to the particulates of the soil medium. The interactions among the three microbial polymers are anticipated to form a composite via multivalent ions and finally end with gels or coacervates in what is called the soil particle assemblage. Other additives such as lignosulfonate, alginic acid, guar gum, etc. may be used for investigation of the soil strength and stability measurements as alternative for xanthan. Molecular weight distribution of the microbial polymers used will be measured by GPC prior to and after interaction. Zeta potential will be determined by streaming potential measurements, and swelling volume measurements will be used to determine the cross-link densities. Finally, we will determine the permeability of the treated soil samples.

Humus materials, which are organic soil fraction or soil organic matter (SOM), occur in natural soil as natural polymers. Those are intermediates leading to geopolymers. They exist in a constant dynamic equilibrium, subject to polymerization, condensation, and degradation processes. These are also referred to as organic soil colloids and are highly associated with the clay portion of the inorganic colloids by charges. The introduction of further biopolymers into the soil material will obviously effect this balance. Fortunately, most biopolymers have certain functional groups. As discussed earlier, the three microbial polymers, chitosan, xanthan, and PHB-PHV can form a semi-penetrating network. They can also participate in network formation. This stable network is at least compatible or exceed to SOM, since many precursors of humin are microbial polymers.

In-situ Production of the Biopolymers

In this task, we will cultivate and grow the three types of microorganisms in a soil environment, determining the optimum conditions for growth. After the cell has grown and has been lysed, we will establish whether or not the polymers will be released, and if so, to what extent. We will refer to some of our previous work in creating the procedures for this task. Specifically, *Alcaligenes* will be assessed for slime-producing species, Xanthan and Chitosan will be isolated in the soil, and *Mucor mucedo* fungus will be grown. Also, the sources of nutrients for the biopolymers in the subsurface will be determined. It is important to study chemotaxis in this task to resolve whether or not the microorganisms can migrate.

In our earlier work, which was supported by the NSF and Navy, we have shown that PHB can be obtained from *Alcaligenes eutrophus*, which is grown in soil matrices. PHB can be obtained from more than 50 common prokaryotic organisms and can be identified in different soil environments. Since *Alcaligenes eutrophus* contains high polyester content, British firm Imperial Chemical Industries (ICI) has produced microbial polyester for industrial uses based upon this species.

We will adopt a similar manner to obtain chitin and chitosan. Another British firm, the British Textile Technology Group (BTTG), has produced chitin/chitosan fibers from the micro-fugal *Mycelia* from a culture of *Mucor mucedo*. Since both PHB and chitosan can be produced in industrial scale, we will use the same species of fungi for their growth in soil. *Xanthomonas campestris* has also been grown in soil to form xanthan and thus, we are very familiar with such processes. All of the biopolymers described here are very inexpensive and many of them are commercially available in large quantities. In addition, lignosulfonate can be obtained from paper wastes, aliginates from kelp, guar gum from beans, etc., all of which are common resource compounds for supplementary studies.

The aim of this task is to produce microbial polymers in soil media. Apparently, according to the known cultivation procedure, no problem has been presented for seed microorganisms to grow, propagate, or transport in the surface of soil particle assemblages. Studies on nutrient requirements are important due to the chemotaxe and the spreading of microorganisms. Nutrient gradients can guide the passage of microorganisms. The inexpensive resources, such as waste sugar or lechate from waste dumps, etc. can become carbon sources.

Prolonged and Enhanced Stability Testing

An environmental chamber will be built and enzymes will be introduced and studied in this task. The samples will be removed from the chamber and the stability of the physical and mechanical properties will be tested after the aging experiment. This will include X-ray photoelectron spectroscopy (XPS) and X-ray diffraction.

The environmental chamber will be constructed in order to study the acceleration in degradation of the biopolymer complexes (the composite) and biopolymers within the soil matrices. High amounts of enzymes will be introduced for close contact with the biopolymers. The chamber can also be heated for simulation of fermentation. One of the goals of using this accelerated aging environmental chamber to examine the formation of geological polymers. Usually, the early stage of kerogen formation can uptake a number of metal species, even uranium.

One of the major points of concern is the biodegradation of the biopolymers used for this project. Chitosan may be an antibacterial agent, which inhibits the growth of microorganisms. However, the PHB type of polymers will be biodegradable. If PHB is entrapped in complexes in the network, such as chitosan-xanthan, then the extent of degradation will be greatly minimized. Most bacterial-produced polyesters are not homopolymers of PHB, but rather random copolymers of PHB and PHV (poly-3-hydroxyvalerate). Both PHB and PHV contain -CH_2—C(=O)--, which can be enolized readily. Not only can the keto function be the site for hydrogen bonding, but the double bonds created after enolization can be cross-linked sites as well. For small amounts of cross-linking, Young's modulus of matrices will change drastically. Since silica and silicate form a great variety of polymeric species and silica is a major component in soil, it can assume hydrogen bonding around a silicon atom, forming a network to enhance soil strength, such as in the admixture of cement before concrete is set. The bonding between the polyester and Si-containing molecules can be identified and quantified. X-ray photoelectron spectroscopy (XPS) will be used as a tool for this investigation.

It is well known that simple alcohols such as glycol, glycerin, and glycol esters can expand the clay sheet. It is necessary to determine whether the bacteria-obtained polyester can also expand the layers of clay sheets. According to the USDA textural triangle or the group symbols of unified soil classification, clay is an essential entity of soil. Clay colloids play an important role in bonding with SOM. If PHB-PHV can reside in clay layers, the soil strength will be greatly reinforced. The tool used in this investigation will be X-ray diffraction. Using x-ray diffraction, we will be able to verify any physical alterations to the structure by biopolymers.

The following is a brief description of our goals in Phase II:

Develop Protocol for Field-testing

The objective of this task is to develop a protocol for field testing, including the formulation of an appropriate suspension for dispensing the polymers into the testing cores. We will conduct an investigation of plugging mechanisms, including examinations of Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) studies. To evaluate any changes in soil permeability after the polymers have been applied, we will utilize the falling head permeability method. A system will be designed and fabricated to deliver the microorganisms into the testing strips and a post-analysis of several regions of the vertical core samples will be conducted.

Perform In-Situ Field Test

During this task, we will conduct baseline characterization of the testing sites, including determination of porosity, permeability, metal speciation and concentration. Field monitoring equipment, such as submersible permeability units, will be obtained and gel delivery suspensions will be prepared. The mixed species will be inoculated with appropriate nutrients and other agents into the soil at the site. The delivery will be performed in such a way that hole-to-hole communication is possible following the growth of the microorganisms. Permeability changes will be continuously monitored through various means to assist in the effort. Soil erodibility will be determined after delivery using the SCS dispersion test, Crumb test, and pin hole test, and the results will be correlated. The permeability will be monitored continuously for 4-6 weeks, followed by soil strength measurements and metal leaching determination.

Results of Previous Projects

The project was just initiated this year. Some of our previous projects have provided additional data that can be utilized in this project, however, and so we have included summaries of those results here:

Zonal bioremediation

PHB-PHV was found to be successful as a plugging agent (see Figs. 3 and 4). We utilized a flow system for the laboratory setup (see Fig. 5). PHB-PHV was made in soil by inoculation of *Alicaligenes eutrophus*. We may use a very inexpensive nutrient, a landfill leachate, to form PHB-PHV (see Fig. 6). A plugging test was implemented, utilizing a sand matrix and *A. eutrophus* cell suspension for plugging. The two simulated subsurface environments were created using both static drainage flow system and pressurized pumping flow system. The results indicated that water permeability was reduced one million fold (refer to Fig. 4). We discovered that microbially-derived polymers can be efficiently used as binding agents to help the soil matrix become stronger and less permeable, therefore restricting the migration of hazardous leachates. It was also discovered that biopolymers are able to prevent fluid migration by promoting the generation of biopolymer-filled soil layers to create a capsule around the spill, similar to slurry wall. See Fig. 7 for a diagram of subsurface zonal bioremediation.

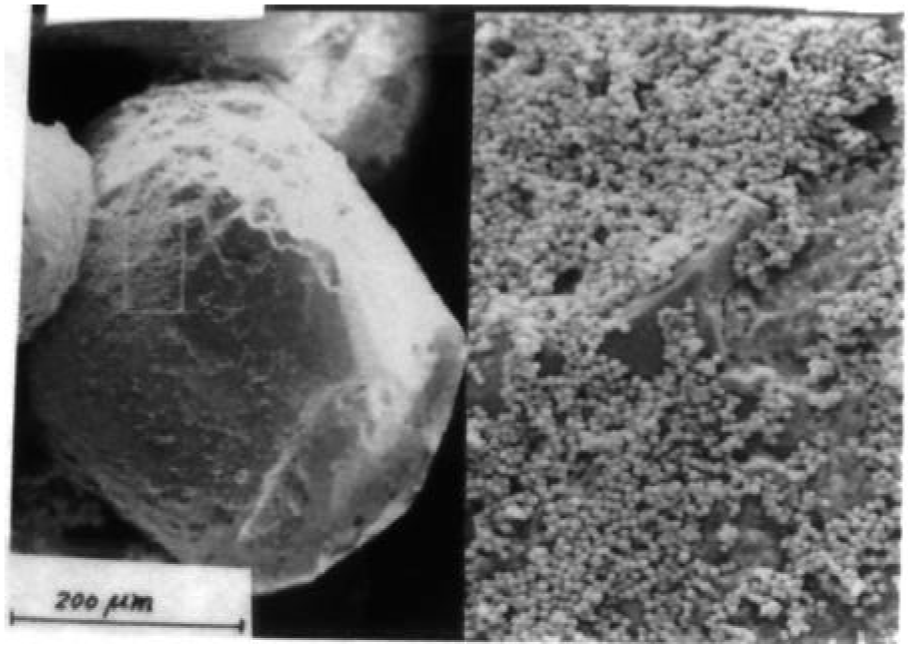


Figure 3. PHB and sand.

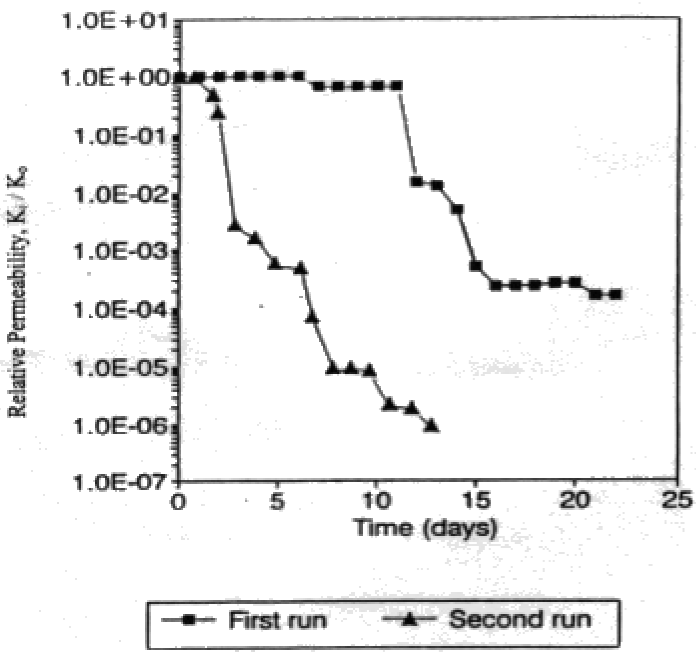


Figure 4. Plugging effect of *A. eutrophus* cells suspension in sand pack columns determined in pressurized flow system. (1) first run; (2) second run. The suspension were 2-day growth cultures and were replaced after 3 days of recycle in the system. (Li, Yang, Lee, and Yen in *Microbial Enhanced Oil Recovery – Recent Advances*, E.T. Premuzic and A. Woodhead Eds., Elsevier, Amsterdam, 1993, pp. 65-77).

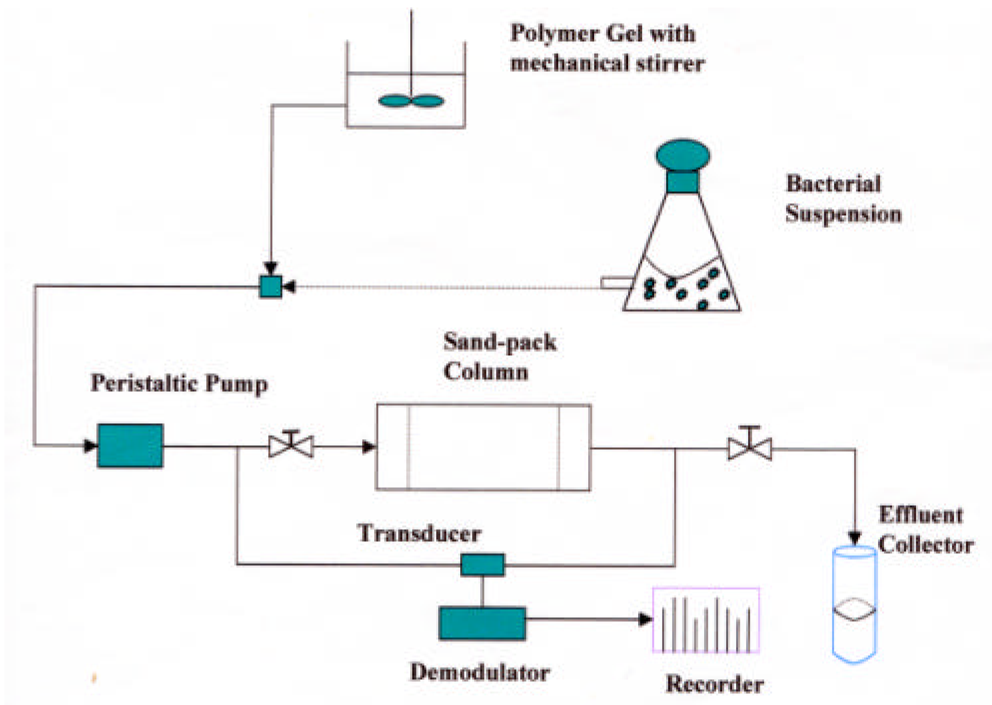


Figure 5. Schematic diagram of the flow apparatus using a peristaltic pump to supply a constant flow. (Either a polymer gel or a bacterial suspension can be introduced).

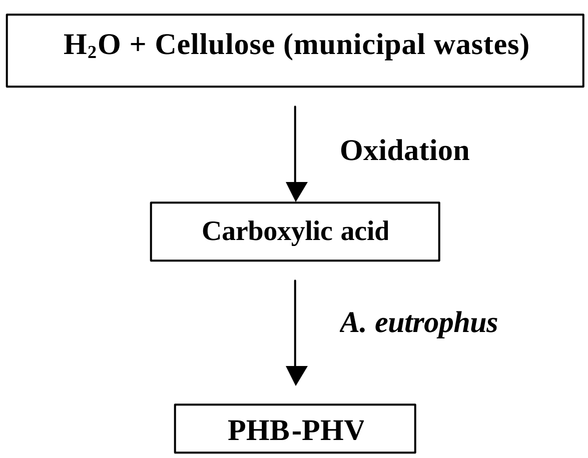


Figure 6. How to make PHB-PHV.

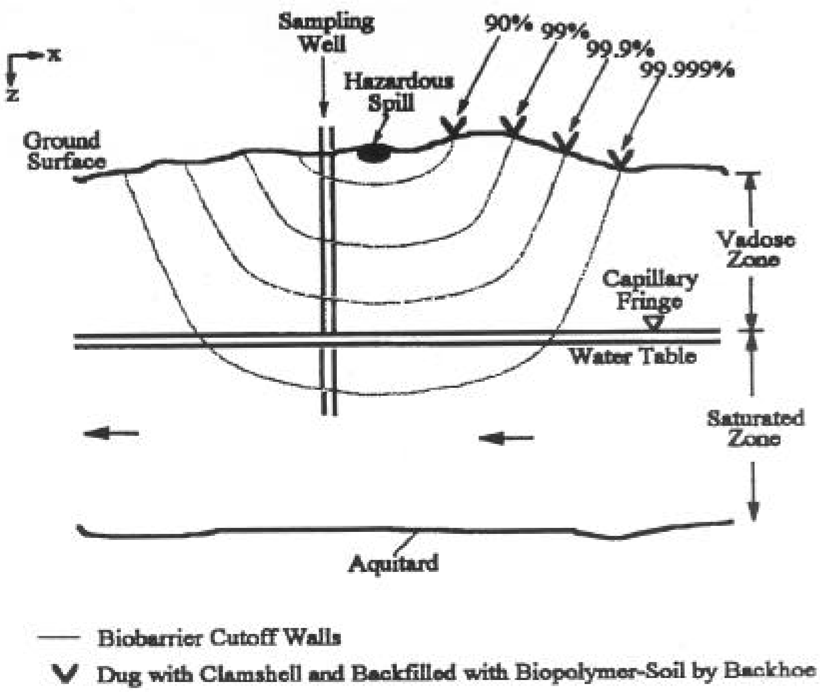


Figure 7. Subsurface zonal bioremediation (T. F. Yen, Bioremediation and Biorestoration, Figure 29-32, *Environmental Chemistry: Chemical Principles by Environmental Processes*, Vol. 4B, Prentice Hall, Upper Saddle River N. J., 1999, p. 1351.

Landfill Liner

Xanthan has been used with sand to enhance soil strength and reduce water permeability. As indicated in Fig. 8, 1% of xanthan in sand can decrease water permeability by four orders of magnitude. The K value has been reduced from 10^{-3} cm sec^{-1} to 10^{-7} cm sec^{-1} (see Fig. 9).

Commercial grade geosynthetic clay layers (GCL) are thin, easy to install and have low permeability. However, GCL suffers because sodium bentonite will change into calcium bentonite via ion exchange, consequently resulting in an increase in water permeability of about 10,000 fold in four years. In addition, more than 50% of shear strength of the bentonite layer will be lost upon hydration (over 40% moisture) (see Fig. 10). The layer made from sand and xanthan, however, will not produce such results (see Fig. 11).

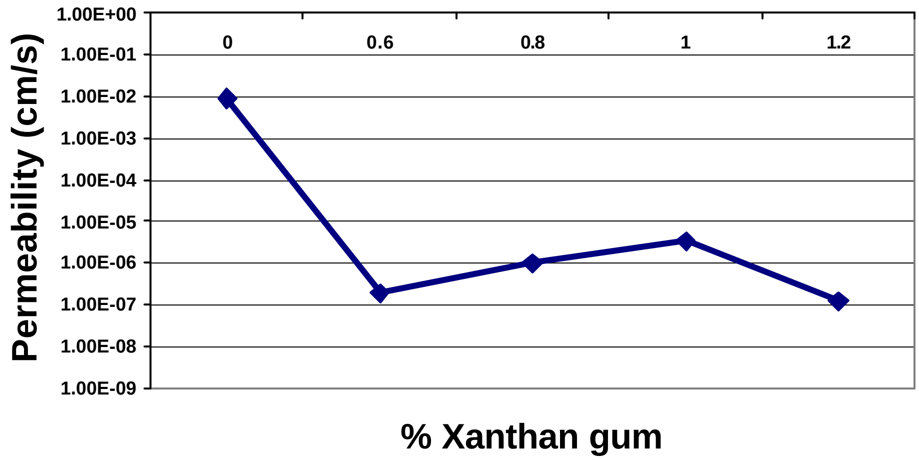


Figure 8. Determination of water permeability of xanthan.

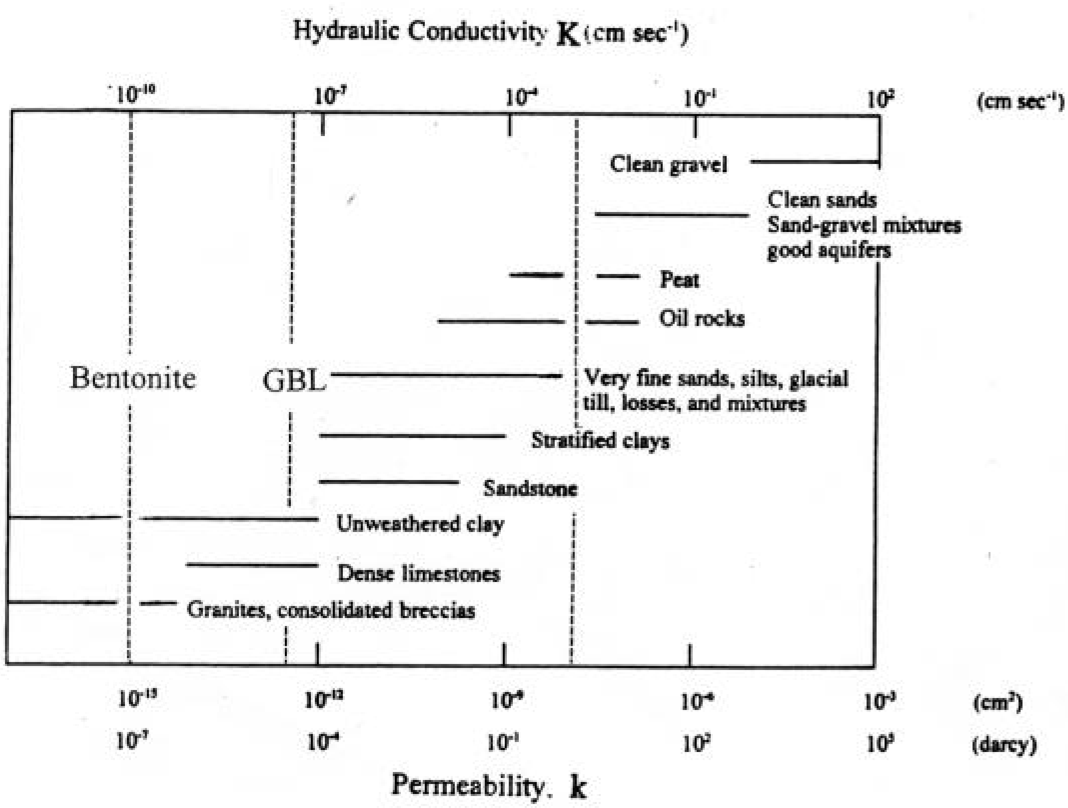


Figure 9. The coefficients of hydraulic conductivity and permeability of sediments and rocks.

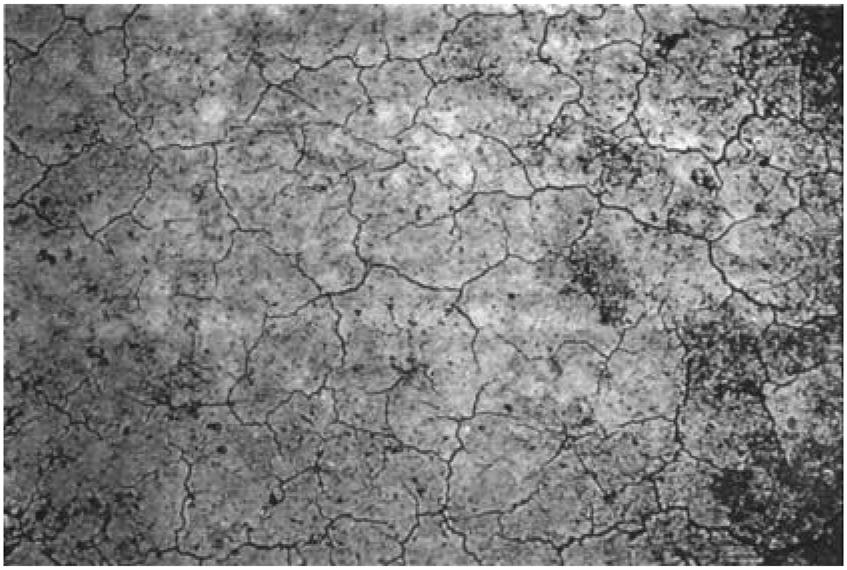


Figure 10. Macro-pores in clay of landfill liner.

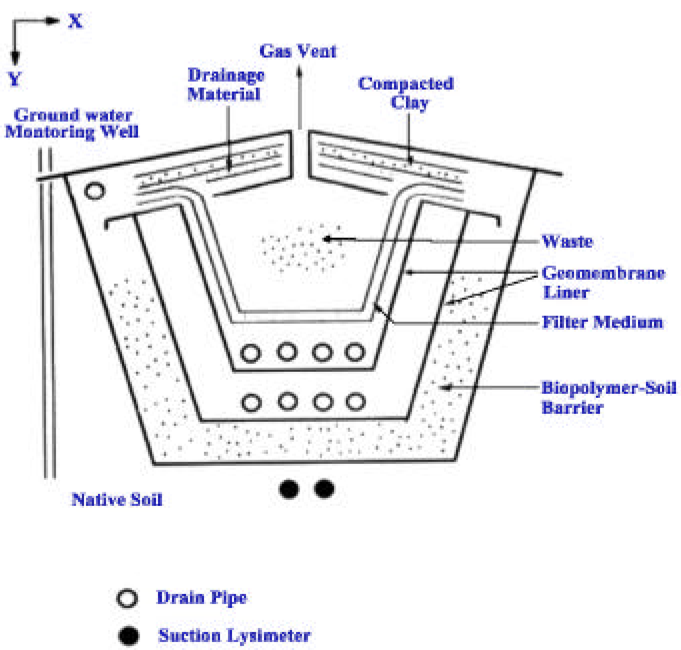


Figure 11. Hazardous waste landfill encapsulation

Erosion Control

Previous work has dealt with surface caps, hanging walls, funnel and gate systems, encapsulating coatings, liners and covers. We have measured the interaction of biopolymers with soil, and consistently found that the soil strength increased for both the Torvane test and unconfined compression test. For example, see Fig. 12 and Table 1 illustrating silica with and without PHB.

Some of USC's work with strength parameters is listed in Table 2. A proposed model for soil interaction is presented in Fig. 13. There are chemical bonds formed just as the bonding between a superplasticizer and aggregates or superplasticizer and portland cement particles for admixture in construction engineering.

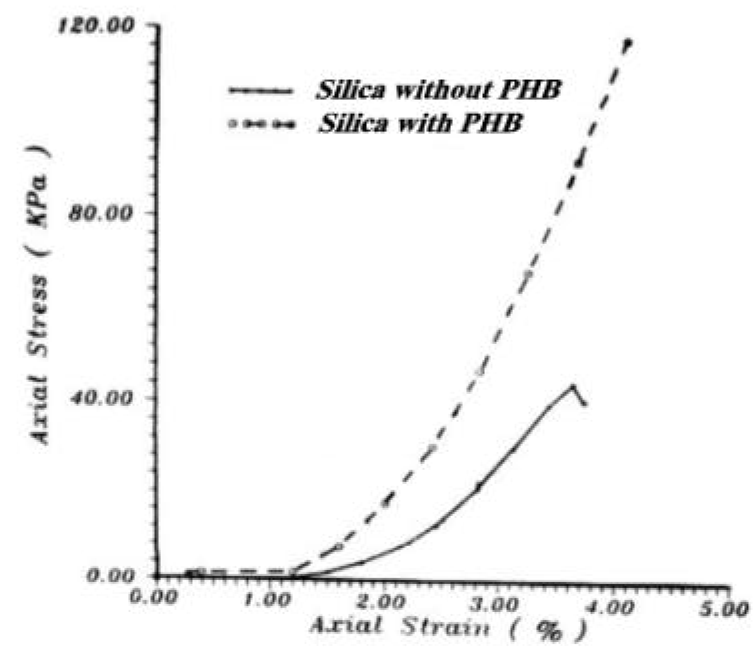


Figure 12. Unconfined compression test.

Table 1. Soil strength evaluation.

Porous media	Treatment process	Ratio of sample stress to control
Silver sand	PHB	1.27
	Humic material (pH = 4)	1.96
	Humic material (pH = 13)	1.96
	Humic material (pH = 1)	0.61
	Distilled water & *A. faecalis*	1.19
	Nutrient Broth	1.21
	Biopolymer from Nutrient Broth & *A. faecalis*	1.26
	Xanthan gum	2.84
	Distilled water & *X. campestris*	1.67
	YMBA Medium	1.42
	Biopolymer from YMBA & *X. campestris*	2.93
Bonnie silt	PHB	2.64
	Xanthan gum	2.47
	Distilled water & *A. faecalis*	1.17
	Distilled water & *X. campestris*	1.85
	Nutrient broth	1.15
	Biopolymer from Nutrient Broth & *A. faecalis*	1.51
	YMBA Medium	1.24
	Biopolymer from YMBA & *X. campestris*	2.75
Bentonite clay	PHB	1.27
	Xanthan gum	1.58
	Distilled water & *A. faecalis*	0.95
	Nutrient broth	1.04
	Biopolymer from Nutrient Broth & *A. faecalis*	1.62
	Distilled water & *X. campestris*	1.09
	YMBA Medium	1.07
	Biopolymer from YMBA & *X. campestris*	1.76

Table 2. Previous USC work with strength parameters.

Soil / Rock Type	Strength parameter C (kg/m^2)
Granite	0 = C = 13
Quartz diorite	Decomposed, sandy, Silty C - 0.1
Diorite	Weathered C = 0.1
Keuper Marl	Highly weathered C = 0.1 Inter.weathered C = 0.1 Unweathered C = 0.3
London clay	Weathered 0.1 = C = 0.2 Unweathered 0.9 = C = 1.8
Gneiss	Decomposed fault zone C = 1.5 Much decomposed C = 4.0 Medium decomposed C = 8.5 Unweathered C = 12.5
Sand (USC)	0.14 = C = 3.0
Silica (USC)	2.40 = C = 3.3

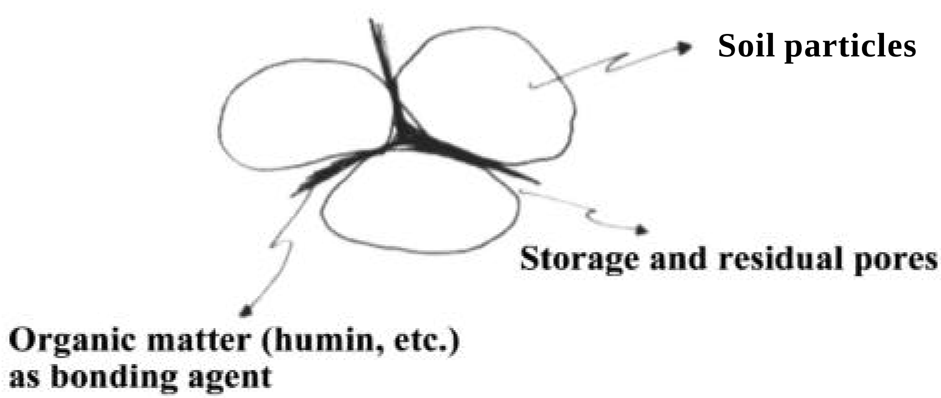


Figure 13. Soil aggregates.

Liquefaction Mitigation

The volume of loose saturated sands tends to diminish under strains or shocks, causing an increase in pore pressure and a decrease in effective stress within the soil. Liquefaction occurs when the pore pressure becomes equal to the effective stress, and the sand consequently loses all its strength and bearing capacity. If liquefaction occurs on an incline, an earthquake will easily set the entire mass in motion as a flow slide of loose, cohesion-less materials. Under such conditions, large hydraulic complexes such as dams must be implemented to bear the heavy load and avoid collapse.

The method of application of polymer gel is as by grout curtain daily sequences (refer to Fig. 14). An example of an experiment using cyclic shear test is illustrated in Fig. 15. There is a small increase in resistance when this result is compared to the impact of the Bonnie Silt sample.

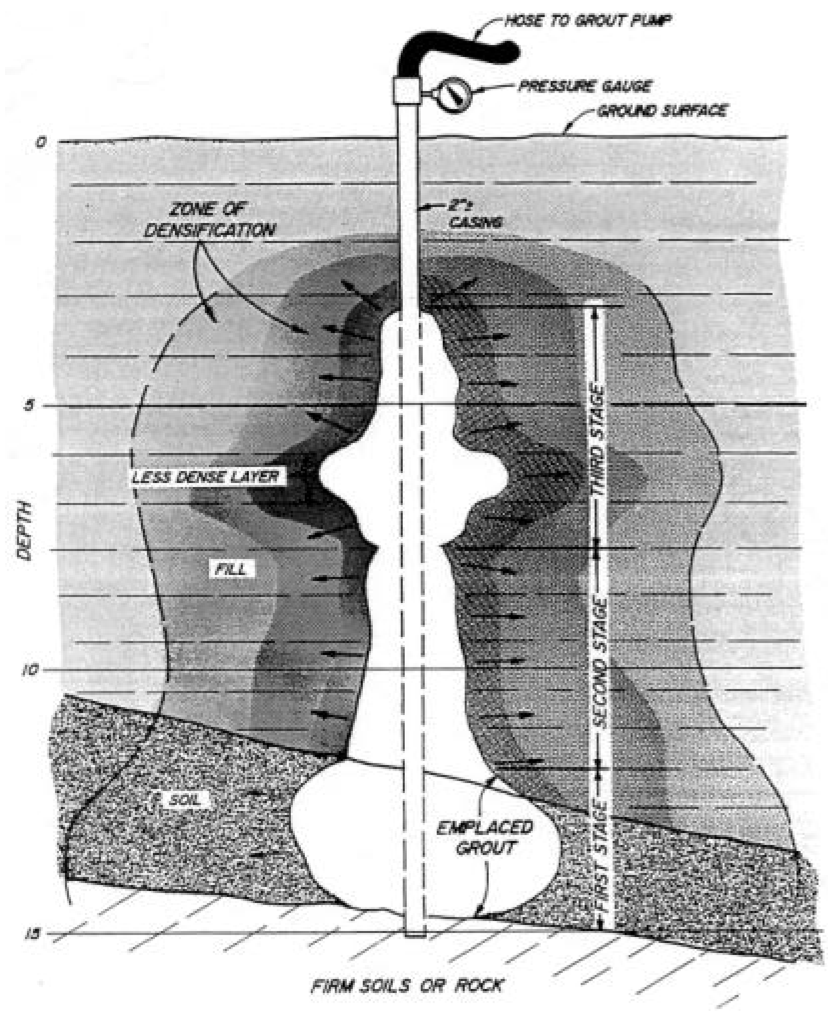


Figure 14. Hypothetical section of idealized irregular grout curtain showing states and approximate zone of influence.

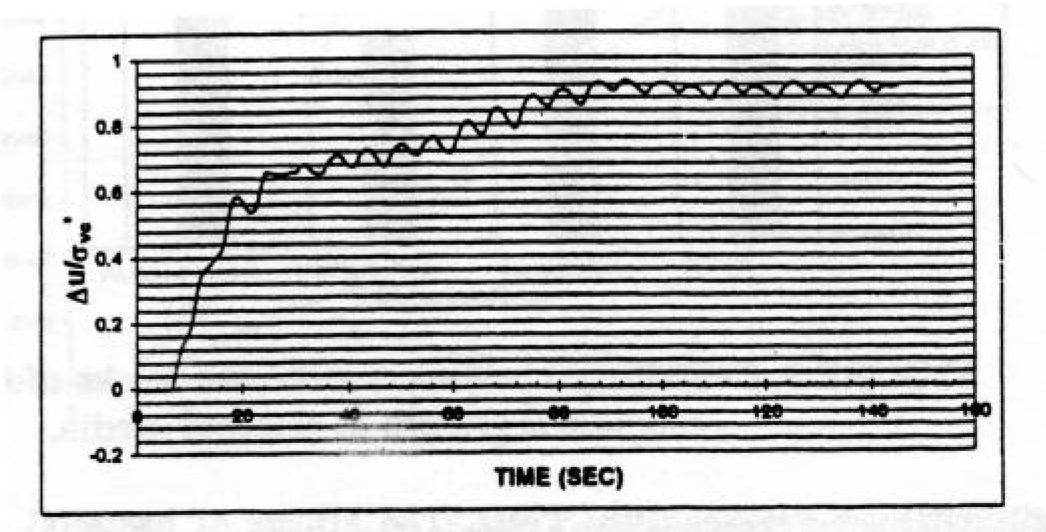


Figure 15. Cyclic simple shear test for compacted Bonnie silt mixed with *Xanthomonas campestris* culture media.

Future Application

The future applications for microbial polymers are unlimited. A few examples have been described in previous sections. With DOE's project, we are optimistic that the ability to fixate and stabilize metal waste will be achieved.

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